Crop Protection 30 (2011) 1297–1305

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Connection between primary *Fusarium* inoculum on gramineous weeds, crop residues and soil samples and the final population on wheat ears in Flanders, Belgium

S. Landschoot [a,b,*], K. Audenaert [a,c], W. Waegeman [b], B. Pycke [a], B. Bekaert [a], B. De Baets [b], G. Haesaert [a,c]

[a] Department of Biological Sciences and Landscape Architecture, University College Ghent, Valentin Vaerwyckweg 1, BE-9000 Ghent, Belgium
[b] KERMIT, Department of Mathematical Modelling, Statistics and Bioinformatics, Ghent University, Coupure links 653, BE-9000 Ghent, Belgium
[c] Department of Crop Protection, Laboratory of Phytopathology, Ghent University, Coupure links 653, BE-9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 30 September 2010
Received in revised form
16 May 2011
Accepted 21 May 2011

Keywords:
Fusarium head blight
Primary inoculum
Winter wheat

ABSTRACT

Fusarium head blight (FHB) is a devastating wheat disease and is influenced by weather conditions and agronomic factors. Since FHB is a mostly monocyclic disease, the quantity of primary inoculum is a key factor influencing its incidence. To investigate the connection between the primary *Fusarium* inoculum and the final population on wheat ears, naturally occurring populations of *Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium poae* and *Microdochium nivale* were studied at eight locations in Flanders, Belgium during the growing seasons of 2008–2009 and 2009–2010. To determine the composition of the primary inoculums in November, weeds, wheat and maize residues as well as soil samples were examined. At the end of the growing season, in July, the population on wheat ears was determined. In both growing seasons, the population was characterized by a large complexity and a differential composition at each location and for each type of sample. Nevertheless, some clear correspondences were observed: *F. culmorum* was a predominant species in crop residues and in soil samples in November, while the population on wheat ears in July consisted mainly of *F. graminearum* and *F. poae*, with only a lower frequency of *F. culmorum*, indicating that soil is not an important source of primary inoculum. The presence of *M. nivale* was restricted to weeds, crop residues and soil samples in November, and was nearly absent in July at the majority of locations. Finally, our results also indicate that the *Fusarium* population in July is more complex than the population at the beginning of the season in November. The information of the primary inoculum and the composition of the FHB population at the end of the growing season is important to predict FHB incidence and to implement control strategies for FHB.

1. Introduction

Fusarium head blight (FHB) is one of the most important diseases in small grain cereals, caused by a complex of *Fusarium* species. Although FHB may cause grain yield losses, the interest in FHB is primarily fueled by the ability of the majority of the *Fusarium* species to produce mycotoxins. These secondary fungal metabolites can accumulate to significant doses and as such cause a serious impediment for human and animal health. A total of 17 species of *Fusarium* have been described to be potentially associated with FHB symptoms (Leonard and Bushnell, 2003). The main causal agents of FHB in Europe are *Fusarium graminearum* Schwabe, *Fusarium culmorum* (Wm. G. Smith) Sacc., *Fusarium avenaceum* (Fr.:Fr.) Sacc., *Fusarium poae* (Peck) Wollenw. and *Microdochium nivale* (Fr.:Fr.) Samuel & I. C. Hallett. *F. graminearum*, *F. culmorum*, *F. avenaceum and F. poae* can produce a plethora of mycotoxins, whereas apparently *M. nivale* does not produce mycotoxins (Xu et al., 2005).

Many laboratories have devoted considerable effort to unequivocally delineate *Fusarium* populations, but the dynamics within a population seems not well studied. Results obtained by Waalwijk et al. (2003) in The Netherlands illustrate the highly dynamic nature of the FHB population with shifts from *F. culmorum* in the early 1990s to *F. graminearum* in 2000 and 2001. In Flanders, Belgium, a similar phenomenon was described by Isebaert et al. (2009) and Audenaert et al. (2009), illustrating a shift from

* Corresponding author. Department of Biological Sciences and Landscape Architecture, University College Ghent, Valentin Vaerwyckweg 1, BE-9000 Ghent, Belgium. Tel.: +32 9 248 88 66; fax: +32 9 242 42 93.
E-mail address: sofie.landschoot@hogent.be (S. Landschoot).

0261-2194/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2011.05.018

a population dominated by *F. graminearum* and *F. culmorum* to a population mainly consisting of *F. poae*. Analogous results were obtained in *Fusarium* surveys throughout Europe (Kosiak et al., 2003; Köhl et al., 2007). Besides these interseasonal variations, Xu et al. (2005) demonstrated the increasing complexity of the population during one growing season from anthesis to harvest by the increasing number of FHB pathogens and the increase of interactions between species. The dynamic nature of the population and the various factors that contribute to this complexity are a serious hindrance to forecasting and controlling FHB during the growing season.

The distribution and predominance of FHB pathogens are to a large extent determined by climatic factors, in particular temperature and moisture. However, under the European climate, these are not the sole variables determining the population's composition. Cropping practices also have an important influence on the predominance of FHB pathogens. Fungicide treatment, crop rotation system, weed management, host resistance and soil tillage are probably the most important agricultural factors governing the outcome and structure of the FHB population (Koch et al., 2006; Schaafsma and Hooker, 2007). In the recent years, major changes could be observed in these cropping practices. Soil tillage systems shifted to minimum or zero tillage to sustain good soil structure and to avoid water and wind erosion. Weed management was redirected to increase ecological diversity by a modified mowing strategy of the surrounding verges. A structured view on the impact of reduced tillage measures and the abundance of gramineous weeds on the composition of primary FHB inoculum remains to be determined. However, reduced tillage measures combined with crop residues overwintering on the field certainly contribute to the primary inoculum in the following cereal crop in spring, since head blight epidemics are mostly monocyclic.

Regarding gramineous weeds it is known that most *Fusarium* species have a broad spectrum of hosts among gramineous weeds. *Fusarium* species residing saprophytically on gramineous weeds could therefore contribute to the primary inoculum. *Fusarium* spp. and *M. nivale* infect wheat ears primarily during anthesis. Chances for infection depend on many factors, including the amount of spores produced in the crop residues and transported to the wheat ears, weather conditions and susceptibility of the wheat variety.

The main sources of inoculum consist of splash-dispersed conidia that originate from infected residues of previous crops still present in the field. A study by Pereyra and Dill-Macky (2008) showed that *F. graminearum* isolates surviving during winter on weeds, such as *Lolium*, *Digitaria* and *Setaria*, were also virulent on wheat and barley. This study also illustrated discrepancies in survival capacities between several species such as *F. graminearum* and *F. poae*. Additionally, *F. graminearum*, *M. nivale* and *F. avenaceum* are able to produce ascospores, which contribute to the local inoculum. These ascospores may also travel longer distances, so that airborne inoculum produced outside the field may also initiate disease (Köhl et al., 2007). Finally, some *Fusarium* species can also persist in soil as saprophytic mycelium or as thick-walled resting spores (chlamydospores).

In this study, the connection between the primary *Fusarium* inoculum on weeds, crop residues and soil samples and the final FHB population on wheat ears was investigated in Flanders, Belgium during the growing seasons 2008–2009 and 2009–2010. Under low infection pressure, a direct relationship between the diversity of inoculum and the disease incidence can be expected because FHB epidemics are mostly monocyclic (Köhl et al., 2007). Knowledge of the correlation between the primary *Fusarium* inoculum and the FHB population can be important to develop a prediction model for FHB.

2. Materials and methods

2.1. Sampling during the growing season

In order to gain a better insight into the *Fusarium* population during the growing seasons 2008–2009 and 2009–2010, samples were taken at Bottelare, Koksijde, Linter, Poperinge, Tongeren, Verrebroek, Zwalm and Zwevegem. These locations are distributed throughout the main wheat regions in Flanders, and are part of the ACC trial network (Agricultural Center Cereals, Roeselare-Beitem, Belgium). At each location, 12 commercial winter wheat varieties were planted in a completely randomized block design with 15 m^2 plots and four replications. At all locations, wheat was produced under normal cropping conditions for Flanders, such as plowing, 350 kernels/m^2 sowing density, three times of N-fertilization and one or two fungicide treatments applied at growth stage (GS) 39 and 59 (Zadoks et al., 1974) to control leaf and other ear diseases and to facilitate the observation of *Fusarium* infection. The fungicide combinations used were the same at all locations and were not specifically directed to *Fusarium* spp by using active ingredients with no specific activity against *Fusarium* spp. and *M. nivale* or by spraying before fully ear emergence. To study the composition of the primary inoculum, at the time of sowing winter wheat (November to December), at each location soil samples, crop residues and weed samples from the field and its surrounding verges were taken. Per wheat variety and replication two soil samples were taken, which resulted in 96 samples per location. From the surrounding verges a range of gramineous weeds, which act as alternative hosts for *Fusarium* species, were determined using the Flora of Weeda et al. (1983). These weeds are Italian ryegrass (*Lolium multiflorum*), perennial ryegrass (*Lolium perenne*), wild oat (*Avena fatua*), annual bluegrass *(Poa annua*), rough meadow grass (*Poa trivialis*), velvet grass (*Holcus lanatus*), cocksfoot (*Dactylis glomerata*), loose silky-bent (*Apera spica venti*), quack grass (*Agropyrum repens*) and common bent (*Agrostis capillaris*). The crop residues include maize and wheat residues, since these are host plants on which *Fusarium* species can survive saprophytically. At each location, 16 weed samples and 16 crop residues (in the case there were crop residues present) were taken.

To determine the composition of the *Fusarium* population during the growing season, samples were taken at the beginning of July, between GS 71 (watery ripe) and GS 75 (milky ripe). At each location, two symptomatic ears from each variety and each replication were harvested. From each ear, two symptomatic kernels were isolated for further identification, resulting in 96 samples per location.

2.2. Plating experiments

The soil samples were air-dried and stored at 4 °C until processing. Subsamples of the soil (10 g each) were added to 100 ml water and mixed thoroughly. One hundred μl of the final dilution was transferred to petri dishes containing dichloran-diglycerol (DG18) agar (Oxoid, Belgium) with 2.5 mg/l Malachite Green Agar (MGA 2.5) and 300 mg/l chloramphenicol. MGA combined with antibacterial agents is a selective medium for isolation of *Fusarium* species (Castellá et al., 1997). The petri dishes were incubated at 20 °C, since at this temperature *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *M. nivale* are able to grow in vitro (Hudec and Muchová, 2010; Brennan et al., 2003). All *Fusarium* colonies were transferred to PDA (potato dextrose agar, Oxoid, Belgium) medium for further species identification. Weeds, crop residues and seeds were surface-sterilized for 1 min in 1% NaOCl, washed for 1 min with 70% EtOH, washed with distilled sterile water, dried for 5 min and subsequently put on PDA plates. After

five days of incubation at 20 °C, outgrowing mycelium was transferred to a new PDA plate.

2.3. Species determination by a species-specific PCR

For species determination, five mycelium plugs randomly taken from the fully grown PDA plates (Section 2.2) were transferred to liquid GPY-broth (10 g glucose, 1 g yeast extract and 1 g peptone, Oxoid, Belgium) and incubated for five days at 20 °C. After five days, mycelium was transferred to eppendorf tubes, centrifuged for 10 min at 12,000 rpm and then freeze-dried for 6 h at −10 °C and 4 h at −50 °C (Christ Alpha 1-2 LD Plus, Osterode, Deutschland). DNA extraction was performed as described by Audenaert et al. (2009), based on the CTAB (hexadecyl trimethyl ammonium bromide) method, described by Saghai-Maroof et al. (1984). PCR for single species detection was performed in a 25 μl reaction mixture (Demeke et al., 2005).

DNA amplification was performed in an Applied Biosystems GeneAmp PCR system 97000 PCR. Amplicons were separated on 1.5% (wt/vol) agarose gels stained with 0.1 μl ethidium bromide. PCR was validated by including reference strains obtained from the MUCL/BCCM collection in each PCR run: *F. graminearum* MUCL 42841; *F. culmorum* MUCL 555; *F. poae* MUCL 6114; *M. nivale* MUCL 15949; *F. avenaceum* MUCL 6130 (Audenaert et al., 2009; Isebaert et al., 2009).

2.4. Statistical analysis

A chi-squared test was used to compare the prevalence of the main *Fusarium* spp. across locations. Relationships between the species on weeds, residues and wheat were investigated using the Pearson product moment correlation. All data were analyzed using the software R (version 2.10.1) (Gentleman and Ihaka, 1997).

3. Results

The prevalence of the *Fusarium* spp. differed significantly between weed samples, crop residues, soil samples in November and wheat samples in July (P-value < 0.001) and between locations (P-value < 0.001). The species showed no preference for certain weeds. During growing season 2008–2009, in November weed samples were dominated by *M. nivale* (35%), followed by *F. avenaceum* (22%), *F. graminearum* (21%), *F. culmorum* (17%) and *F. poae* (5%), while crop residues were dominated by *F. graminearum* (44%), followed by *F. culmorum* (28%), *M. nivale* (13%), *F. avenaceum* (8%) and *F. poae* (8%). Soil samples were clearly characterized by *F. culmorum* (99%); only a few soil samples contained *F. graminearum* (1%). In July 2009, *F. graminearum* (40%) was most frequently encountered on wheat samples, followed by *F. poae* (29%), *M. nivale* (14%), *F. culmorum* (12%) and *F. avenaceum* (6%).

During the 2009–2010 growing season, remarkable differences were observed for the *Fusarium* population. In November 2009, weed samples were dominated by *F. poae* (38%), followed by *F. culmorum* (30%), *M. nivale* (14%), *F. graminearum* (10%) and *F. avenaceum* (8%). Crop residues were dominated by *F. culmorum* (64%), followed by *F. graminearum* (14%), *F. poae* (14%) and *F. avenaceum* (4%), while *M. nivale* was not detected on crop residues. Different from the 2008–2009 growing season, in 2009–2010 all five studied *Fusarium* species were detected in the soil samples, but *F. culmorum* (63%) was still the predominant species followed by *F. poae* (25%), *F. graminearum* (5%), *F. avenaceum* (4%) and *M. nivale* (2%). The population in July 2010 was dominated by *F. poae* (37%) followed by *F. graminearum* (35%) and *F. culmorum* (26%); *F. avenaceum* (1%) and *M. nivale* (1%) were rarely detected in July 2009 (Fig. 1).

In order to get a better insight into population shifts between November and July, a closer view was taken at each location separately. Throughout Flanders, in July 2009, *F. graminearum* was detected at each location on wheat ears just before harvest and its presence varied between 26% (Verrebroek) and 59% (Poperinge). When the populations in the primary inoculum and the FHB population at the end of the season were compared, some important differences were found. At first, *F. poae* was not detected in the primary inoculum of Koksijde, Linter, Poperinge and Tongeren, but in July each field contained more than 10% *F. poae*. Thus, weed samples and crop residues were probably not the major sources of the *F. poae* inoculum. Furthermore, *F. culmorum*, omnipresent in soil samples, represented only a small part of the final population. *F. avenaceum* incidence decreased at all locations. The most striking decrease was in Tongeren, from 29% on weeds to 4% on wheat ears. A similar decrease was found for *M. nivale* (except in Tongeren); in Zwalm *M. nivale* was even the main species on crop residues (67%), but on wheat ears in July this species was completely absent. In Bottelare, the population on wheat ears in July was similar to the population on crop residues. Similarly, in Poperinge, the population in July 2009 on wheat ears was similar to the population on weeds

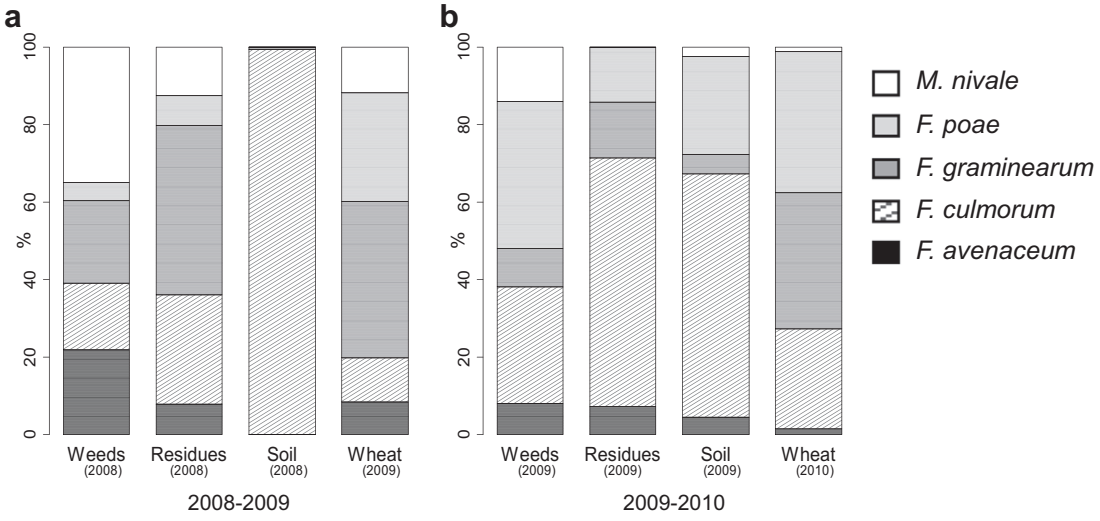

Fig. 1. Composition (%) of the primary inoculum on weed samples, crop residues and soil samples in November and the final *Fusarium* population on wheat ears in July during growing season 2008–2009 (a) and 2009–2010 (b).

in November 2008 and in Zwevegem the population on wheat ears was similar to the population on weeds and on crop residues in November 2008. For the other locations, the populations in the primary inoculum differed from the FHB populations on wheat ears (Fig. 2). During the 2009–2010 growing season, *F. culmorum* was predominantly present in November, which was similar to the result of 2008–2009. The presence of *F. avenaceum* was mainly restricted to November in weeds, crop residues and soil samples. Bottelare, Linter and Verrebroek had a similar population in July 2010 on wheat ears, although the primary inoculum on these locations was different. In Verrebroek, *F. poae* was the only species detected on weed samples. The population on weed samples in Tongeren and Linter was similar with only *F. poae* and *M. nivale*. At the other locations three or more different species were detected on weed samples. Regarding crop residues, maize residues were analyzed in Bottelare and Zwevegem. In Bottelare, only *F. culmorum* and *F. graminearum* were detected, whereas in Zwevegem all five studied *Fusarium* species were present. Still, a correspondence between both locations was observed: *F. culmorum* was the predominant species on maize residues in both locations.

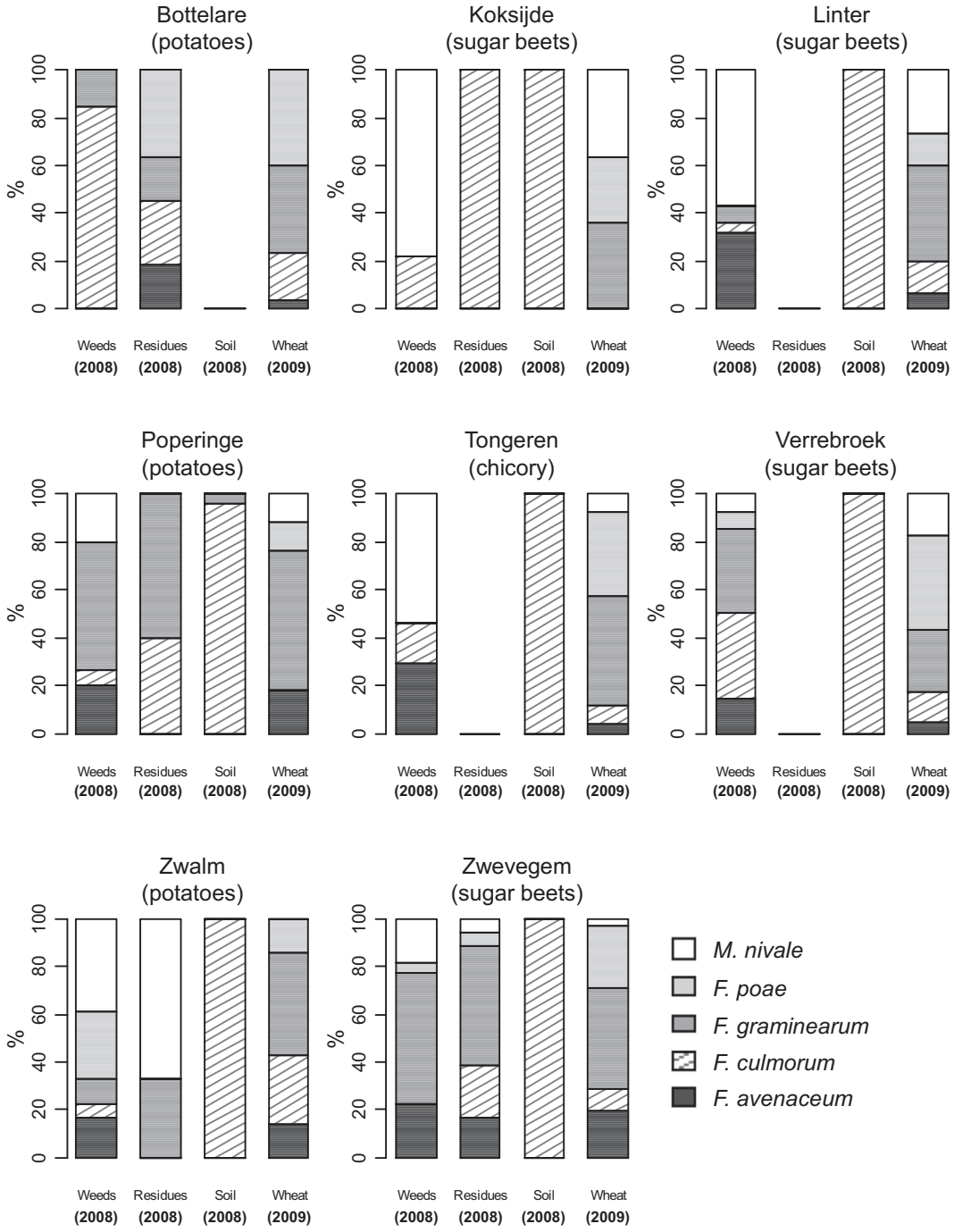

Fig. 2. Composition (%) of the primary inoculum on weed samples, crop residues, soil samples at each location in November 2008 and the final *Fusarium* population in July 2009 (between brackets the preceding crop is indicated). Note that none of the preceding crops were host crops for *Fusarium* species, which means that the crop residues from maize found at the locations date from two years ago.

In Zwalm, *F. poae* was most frequently detected in soil samples, at all other locations, *F. culmorum* was predominantly present. However, it was not the sole species, also all other species were present in the soil samples. In July 2010 on wheat ears, we can see a clear difference between the species composition at the different locations. Notable is the abundance of *F. poae* in Linter, Tongeren, Verrebroek, Zwalm and Zwevegem. Comparing the populations in November and July, an increase of *F. graminearum* at each location and a decrease of *F. culmorum* and *M. nivale* were observed. Remarkably, at the locations with maize as a preceding crop, no clear increase of *F. graminearum* in the subsequent wheat crop was observed. Moreover, *F. graminearum* was only a minor species in Zwevegem in July 2010 on wheat ears. At the locations where *F. poae* was abundantly present in November (Linter, Tongeren, Verrebroek, Zwalm and Zwevegem), it was also abundantly present in July (Fig. 3).

The majority of wheat ears appeared to be colonized by a single *Fusarium* species. Tables 1 and 2 show the percentage of samples with no, one, two or three different *Fusarium* species identified.

During the 2008–2009 growing season, in November, co-occurrence of *F. avenaceum* and *M. nivale* (eight) on single ears was observed, whereas this combination on crop residues was

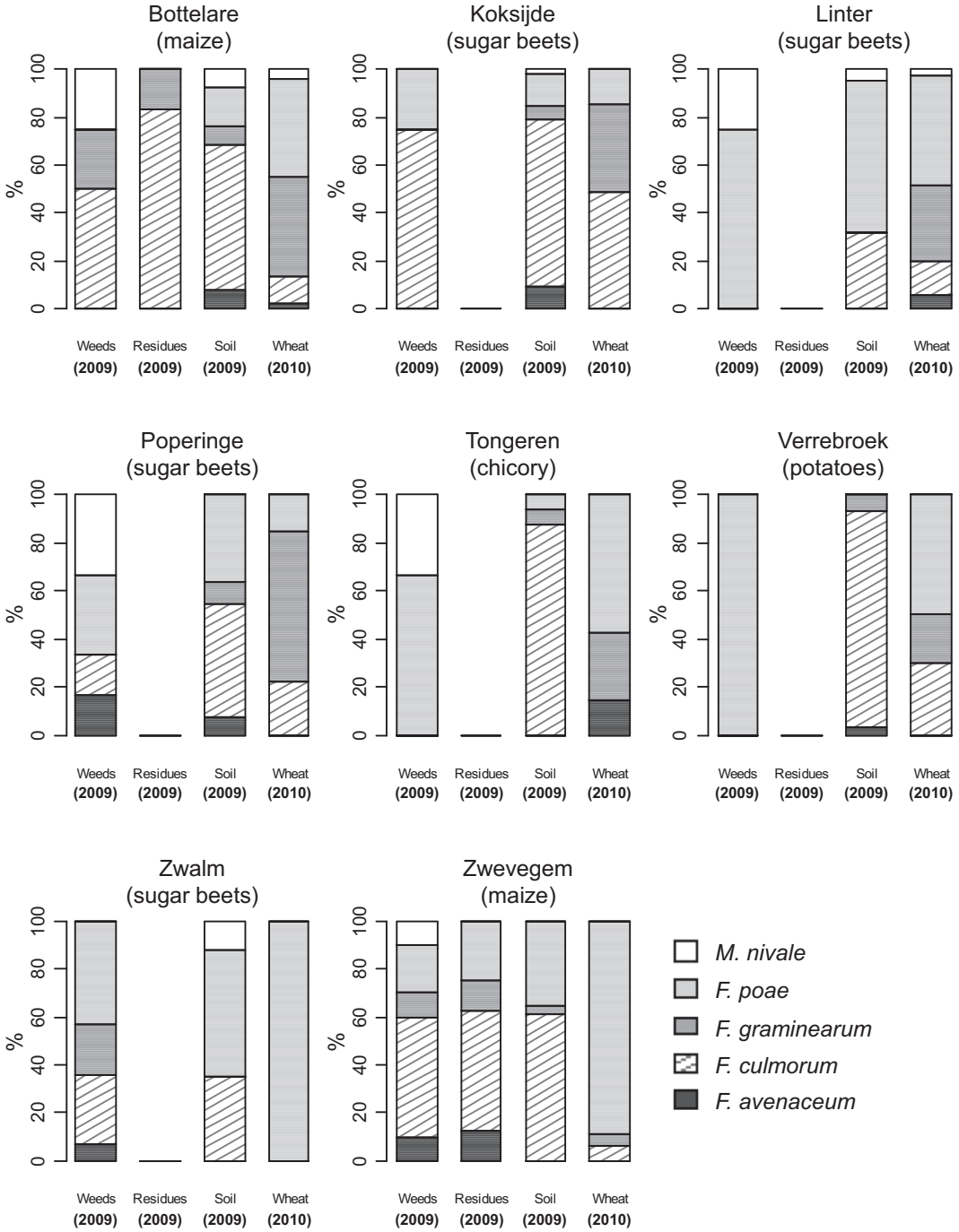

Fig. 3. Composition (%) of the primary inoculum on weed samples, crop residues, soil samples at each location in November 2009 and the final *Fusarium* population in July 2010 (between brackets the preceding crop is indicated).

Table 1
Percentage of weed samples, crop residues and wheat colonized by no, one, two or three *Fusarium* species in the 2008–2009 growing season.

Location	No *Fusarium* spp.			One species			Two species			Three species		
	Weeds (2008)	Crop residues (2008)	Wheat (2009)	Weeds (2008)	Crop residues (2008)	Wheat (2009)	Weeds (2008)	Crop residues (2008)	Wheat (2009)	Weeds (2008)	Crop residues (2008)	Wheat (2009)
Bottelare	76.47	64.52	27.27	21.57	35.48	54.55	1.96	0.00	18.18	0.00	0.00	0.00
Koksijde	43.75	60.00	15.79	56.25	40.00	63.16	0.00	0.00	10.53	0.00	0.00	10.53
Linter	37.84	–	28.57	48.65	–	71.43	13.51	–	0.00	0.00	–	0.00
Poperinge	64.29	60.00	27.78	17.86	30.00	50.00	17.86	10.00	22.22	0.00	0.00	0.00
Tongeren	19.05	100.00	27.59	47.62	0.00	55.17	33.33	0.00	17.24	0.00	0.00	0.00
Verrebroek	53.57	–	26.92	42.86	–	57.69	3.57	–	15.38	0.00	–	0.00
Zwalm	57.14	0.00	9.09	28.57	100.00	54.55	7.14	0.00	36.36	7.14	0.00	0.00
Zwevegem	29.17	15.38	25.93	50.00	38.46	40.74	20.83	38.46	25.93	0.00	7.69	7.41
Mean	47.66	49.98	23.62	39.17	40.66	55.91	12.28	8.08	18.23	0.89	1.28	2.24

never detected. On crop residues, in November 2008, the combination *F. culmorum*–*F. graminearum* was most frequently observed (four). On wheat ears, in July 2009, the number of samples with *F. graminearum* and *F. poae* (16) was clearly higher than the other two-species combinations. In November 2009 only a few weed and crop residue samples contained two different species. On wheat ears, in July 2010, most two-species combinations were detected between *F. poae* and *F. graminearum* (33) (Table 3).

During the 2008–2009 growing season, in November, the co-occurrence of three species was detected on weed samples in Zwalm (co-occurrence of *F. graminearum*, *M. nivale* and *F. poae*) and on crop residues in Zwevegem (co-occurrence of *F. avenaceum*, *F. culmorum* and *F. graminearum*). In July 2009, the co-occurrence of three species on wheat ears was detected in Zwevegem (co-occurrence of *F. avenaceum*, *F. graminearum* and *F. poae*) and in Koksijde (co-occurrence of *F. graminearum*, *M. nivale* and *F. poae*). During the 2009–2010 growing season, in November, the co-occurrence of three species was detected on weed samples in Zwalm (co-occurrence of *F. avenaceum*, *F. culmorum* and *F. poae*) and in soil samples in Poperinge (co-occurrence of *F. culmorum*, *F. poae* and *F. graminearum*). In July 2010, no co-occurrence of three species was detected.

4. Discussion

This field survey in Flanders revealed that the *Fusarium* population varied significantly from location to location, both at the beginning of the season on weeds, crop residues and in soil samples and at the end of the growing season on wheat ears. Regional differences in species profile may exist due to different crop rotation systems and local climatic conditions (especially temperature and precipitation) (Waalwijk et al., 2003; Xu et al., 2005).

The dominance of *F. culmorum* in soil samples at almost all locations is not surprising because *F. culmorum* is a soil-inhabiting fungus (Wagacha and Muthomi, 2007). In addition to FHB, this pathogen also causes seedling blight and foot rot (Bateman, 2005). In this study, *F. culmorum* was detected in almost all of the soil samples of November 2008. However, in November 2009 all five studied *Fusarium* species were detected, even through *F. culmorum* was still the most frequent species in the soil samples. The weed and crop residue samples had much greater diversities in species than the soil samples. On the weed samples of November 2008, *M. nivale* was detected as the major species, while the frequencies of *F. avenaceum* and *F. graminearum* were about the same and not as frequent as *M. nivale*. In November 2008, *F. poae* was a minor species, while in November 2009, it was the most important species on weed samples. On crop residues of both growing seasons, *F. graminearum* and *F. culmorum* were the most important species. This was expected since only residues of major host plants, maize and wheat, were sampled. Maize is an important host for *F. graminearum*, which is able to survive on maize stubble. Champeil et al. (2004), Pereyra et al. (2004) and Pereyra and Dill-Macky (2008) concluded that the highest levels of *F. graminearum* contamination are recorded on grains harvested from wheat crops following maize in rotation. Based on our results, this is an oversimplified conclusion since *F. graminearum* was only a minor species in Zwevegem on wheat ears in 2010. In addition to the effect of previous crops, other factors also influence the composition of the population.

In both growing seasons, *F. graminearum* was the most important species detected on wheat ears. *F. graminearum* was also an important species in Flanders in July 2007 and July 2008 (Audenaert et al., 2009). The importance of *F. graminearum* is in accordance with the reports from other European surveys that point to an increase in the importance of *F. graminearum* as a major

Table 2
Percentage of weed samples, crop residues and wheat colonized by no, one, two or three *Fusarium* species in the 2009–2010 growing season.

	No *Fusarium* spp.			One species			Two species			Three species		
	Weeds (2009)	Crop residues (2009)	Wheat (2010)	Weeds (2009)	Crop residues (2009)	Wheat (2010)	Weeds (2010)	Crop residues (2009)	Wheat (2010)	Weeds (2009)	Crop residues (2009)	Wheat (2010)
Bottelare	33.33	53.85	23.60	66.67	49.12	50.56	0.00	0.00	25.84	0.00	0.00	0.00
Koksijde	0.00	–	26.26	100.00	–	55.00	0.00	–	18.75	0.00	–	0.00
Linter	55.56	–	20.59	44.44	–	55.88	0.00	–	23.53	0.00	–	0.00
Poperinge	42.86	–	25.26	28.57	–	65.26	28.57	–	9.47	0.00	–	0.00
Tongeren	40.00	–	33.33	60.00	–	66.67	0.00	–	0.00	0.00	–	0.00
Verrebroek	66.67	–	54.55	33.33	–	45.45	0.00	–	0.00	0.00	–	0.00
Zwalm	0.00	–	43.75	55.56	–	56.25	33.33	–	0.00	11.11	–	0.00
Zwevegem	50.00	33.33	21.43	44.44	44.44	75.00	5.56	22.22	3.57	0.00	0.00	0.00
Mean	36.05	43.59	31.09	54.13	45.30	58.76	8.43	11.11	10.15	1.39	0.00	0.00

Table 3
Number of two-species combinations on single samples of weeds and crop residues in November 2008 and November 2009 and on wheat ears in July 2009 and July 2010.

Pathogen one	Pathogen two	Number of two-species combinations					
		Weeds		Crop residues		Wheat	
		2008	2009	2008	2009	2009	2010
F. avenaceum	*F. culmorum*	0	1	1	0	2	1
F. avenaceum	*F. graminearum*	6	0	2	0	3	1
F. avenaceum	*M. nivale*	8	0	0	0	1	2
F. avenaceum	*F. poae*	2	2	0	0	4	1
F. culmorum	*F. graminearum*	2	1	4	1	5	23
F. culmorum	*M. nivale*	4	0	0	0	0	9
F. culmorum	*F. poae*	0	2	0	1	3	16
F. graminearum	*M. nivale*	5	0	1	0	6	9
F. graminearum	*F. poae*	2	2	1	0	16	33
M. nivale	*F. poae*	3	1	0	0	4	6

pathogen of wheat in temperate climates (Waalwijk et al., 2003; Isebaert et al., 2009). The factors for this shift have not been elucidated, but an increase in maize area has been suggested to play an important role. Within each growing season, some remarkable differences between the primary inoculum on weeds, crop residues and soil samples and the FHB population on wheat ears were observed. On weed and crop residue samples of November 2008, *F. poae* was a minor species (except in Bottelare and Zwalm), but on wheat ears in July 2009, the frequency of this species varied between 0% and 39%. So, gramineous weeds and crop residues were probably not important sources of the *F. poae* inoculum. However, in 2007 and 2008, *F. poae* was a dominant species (Audenaert et al., 2009). In the present study, this species represented only a small part of the final FHB population found on wheat ears in July 2009. The fact that *F. poae* is more prominent in 2007 and 2008, years with respectively high and moderate infection pressure, would be a consequence of its nature as a secondary pathogen, colonizing the weakened ears already infected by other more aggressive FHB pathogens (Audenaert et al., 2009). In 2009–2010, *F. poae* was abundantly present in the primary inoculum and in the final population on wheat ears. In both growing seasons the frequencies of *M. nivale* and *F. culmorum* species decreased during the growing season and the frequency of *F. graminearum* species rose during the growing season.

On gramineous weeds, crop residues and wheat ears, most pathogens appeared as single species, but in July, on wheat ears, the co-occurrence of multiple species was detected more often than in November in the primary inoculum. This is in line with the observations of Xu et al. (2005), who studied the co-occurrence of two *Fusarium* species in harvest samples. *F. poae* was frequently detected together with other species, especially with *F. graminearum* on wheat ears. This is in accordance with the hypothesis of Audenaert et al. (2009) that *F. poae* mainly acts as a secondary invader. Note, that if no species were detected, it does not automatically mean that no *Fusarium* spp. were present, as there may be other species present that were not included in the PCR reaction, such as *Fusarium langsethiae*. However, no isolates were observed with typical *F. langsethiae* morphology.

This study demonstrated that the FHB population is characterized by a large variability and complexity and a differential population composition at each location and type of sample (weeds, crop residues, soil and wheat ears). The FHB population in July was more complex than the primary inoculum in November, as both the number of different species and the number of samples with different *Fusarium* species rose during the growing season. This can be due to the fact that *Fusarium* species survive saprophytically on crop residues and weeds. During the growing season the complexity might increase due to antagonistic and synergistic interactions between species during the active infection and colonization process of the ears.

One of the hypotheses explaining the differences between the populations in November and July can be the mode of dispersal of the different species. Conidia formed by *F. avenaceum*, *F. graminearum*, *M. nivale* and *F. poae* are typically vertically transported by rain drops. Because these conidia are entrapped in rain drops, they cannot be easily transported by wind. On the contrary, ascospores (from *F. avenaceum*, *F. graminearum* or *M. nivale*) are flinged away from perithecia with enormous force, and can be easily picked up by the wind and transported over long distances (Champeil et al., 2004). So, it is possible that ascospores from other fields contributed to the FHB population or that conidia present in weed samples, crop residues and soil samples could not reach the wheat ears. Our findings highlight the importance of frequent surveys of field epidemics to identify the FHB population. In this study we focused on the species composition of the primary *Fusarium* inoculum in November and the FHB population on wheat ears in July and we studied the five most important *Fusarium* species. There seems to be no clear relationship between the species composition of the primary inoculum on weeds, crop residues and soil samples and the FHB population on wheat ears, but we can see some general trends, such as an increase of *F. poae* and *F. graminearum* during the growing season and a decrease of *F. culmorum*.

Acknowledgments

Geert Haesaert is a full professor at the Ghent University College and coordinates this study. Kris Audenaert is a postdoctoral fellow of the Research Fund of Ghent University College. Willem Waegeman is supported as a postdoc by the Research Foundation-Flanders (FWO Flanders). This work was financially supported by the Agency for Innovation by Science and Technology, project 70575 (IWT, Brussels, Belgium). We thank the ACC Trial Network for putting the wheat samples at our disposal.

Appendix

Table A.1
Overall composition (%) of primary inoculum on weed samples, crop residues and soil samples in November 2008 and the final *Fusarium* population on wheat ears in July 2009; the dominant species are indicated in bold.

	F. avenaceum	*F. culmorum*	*F. graminearum*	*M. nivale*	*F. poae*
Gramineous weeds (2008)	21.89	17.16	21.30	**34.91**	4.73
Crop residues (2008)	7.81	28.13	**43.75**	12.50	7.81
Soil (2008)	0.00	**99.35**	0.65	0.00	0.00
Wheat (2009)	6.12	11.56	**40.14**	13.61	28.57

Table A.2
Overall composition (%) of primary inoculum on weed samples, crop residues and soil samples in November 2009 and the final *Fusarium* population on wheat ears in July 2010; the dominant species are indicated in bold.

	F. avenaceum	*F. culmorum*	*F. graminearum*	*M. nivale*	*F. poae*
Gramineous weeds (2009)	8.00	30.00	10.00	14.00	**38.00**
Crop residues (2009)	7.14	**64.29**	14.29	0.00	14.29
Soil (2009)	4.44	**62.80**	5.12	2.39	25.26
Wheat (2010)	1.43	25.78	35.08	1.19	**36.52**

Table A.3
Composition (%) of primary inoculum on weed samples and crop residues in November 2008 and the final *Fusarium* population on wheat ears in July 2009; the dominant species are indicated in bold.

	F. avenaceum			*F. culmorum*			*F. graminearum*			*M. nivale*			*F. poae*		
	Weeds (2008)	Crop residues (2008)	Wheat (2009)	Weeds (2008)	Crop residues (2008)	Wheat (2009)	Weeds (2008)	Crop residues (2008)	Wheat (2009)	Weeds (2008)	Crop residues (2008)	Wheat (2009)	Weeds (2008)	Crop residues (2008)	Wheat (2009)
Botttelare	0.00	18.18	3.33	**84.62**	27.27	20.00	15.38	18.18	36.67	0.00	0.00	0.00	0.00	**36.36**	**40.00**
Koksijde	0.00	0.00	0.00	22.22	**100.00**	0.00	0.00	0.00	36.36	**77.78**	0.00	**36.36**	0.00	0.00	27.27
Linter	32.14	–	6.67	3.57	–	13.33	7.14	–	**40.00**	**57.14**	–	26.67	0.00	–	13.33
Poperinge	20.00	0.00	17.65	6.67	40.00	0.00	**53.33**	**60.00**	**58.82**	20.00	0.00	11.76	0.00	0.00	11.76
Tongeren	29.17	–	3.85	16.67	–	7.69	0.00	–	**46.15**	**54.17**	–	7.69	0.00	–	34.62
Verrebroek	14.29	–	4.35	**35.71**	–	13.04	35.71	–	26.09	7.14	–	17.39	7.14	–	**39.13**
Zwalm	16.67	0.00	14.29	5.56	0.00	28.57	11.11	33.33	**42.86**	**38.89**	66.67	0.00	27.78	0.00	14.29
Zwevegem	22.73	16.67	19.35	0.00	22.22	9.68	**54.55**	**50.00**	**41.94**	18.18	5.56	3.23	4.55	5.56	25.81

Table A.4
Composition (%) of primary inoculum on weed samples and crop residues in November 2009 and the final *Fusarium* population on wheat ears in July 2010; the dominant species are indicated in bold.

	F. avenaceum				*F. culmorum*				*F. graminearum*				*M. nivale*				*F. poae*			
	Weeds (2009)	Crop residues (2009)	Soil (2009)	Wheat (2010)	Weeds (2009)	Crop residues (2009)	Soil (2009)	Wheat (2010)	Weeds (2009)	Crops residues (2009)	Soil (2009)	Wheat (2010)	Weeds (2009)	Crop residues (2009)	Soil (2009)	Wheat (2010)	Weeds (2009)	Crop residues (2009)	Soil (2009)	Wheat (2010)
Botttelare	0.00	0.00	7.89	2.20	**50.00**	**83.33**	**60.53**	10.99	25.00	16.67	7.89	**41.76**	25.00	0.00	7.89	4.40	0.00	0.00	15.79	40.66
Koksijde	0.00	–	9.62	0.00	**75.00**	–	**69.23**	**48.65**	0.00	–	5.77	36.49	0.00	–	1.92	0.00	25.00	–	13.46	14.86
Linter	0.00	–	0.00	5.71	0.00	–	31.82	14.29	0.00	–	0.00	31.43	25.00	–	4.55	2.86	**75.00**	–	**63.64**	**45.71**
Poperinge	16.67	–	7.27	0.00	16.67	–	**47.27**	22.50	0.00	–	9.09	**62.50**	**33.33**	–	0.00	0.00	**33.33**	–	36.36	15.00
Tongeren	0.00	–	0.00	14.29	0.00	–	**87.50**	0.00	0.00	–	6.25	28.57	33.33	–	0.00	0.00	66.67	–	6.25	**57.14**
Verrebroek	0.00	–	3.23	0.00	0.00	–	**90.32**	30.00	0.00	–	6.45	20.00	0.00	–	0.00	0.00	**100.00**	–	0.00	**50.00**
Zwalm	7.14	–	0.00	0.00	28.57	–	35.29	0.00	21.43	–	0.00	0.00	0.00	–	11.76	0.00	**42.86**	–	**52.94**	**100.00**
Zwevegem	10.00	12.50	0.00	0.00	**50.00**	**50.00**	**61.29**	6.52	10.00	12.50	3.23	4.35	10.00	0.00	0.00	0.00	20.00	25.00	35.48	**89.13**

References

Audenaert, K., Van Broeck, R., De Witte, F., Heremans, B., Messens, K., Höfte, M., Haesaert, G., 2009. Fusarium head blight (FHB) in Flanders: population diversity, inter-species associations and DON contamination in commercial winter wheat varieties. Eur. J. Plant Pathol. 125, 445–458.

Bateman, G.L., 2005. The contribution of ground-level inoculum of *Fusarium culmorum* to ear blight of winter wheat. Plant Pathol. 54, 299–307.

Brennan, J.M., Fagan, B., van Maanen, A., Cooke, B.M., Doohan, F.M., 2003. Studies on in vitro growth and pathogenicity of European *Fusarium* fungi. Eur. J. Plant Pathol. 109, 577–587.

Castellá, G., Bragulat, M., Rubiales, M., Cabañas, F., 1997. Malachite green agar, a new selective medium for *Fusarium* spp. Mycopathologia 137, 173–178.

Champeil, A., Doré, T., Fourbet, J.F., 2004. Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. Plant Sci. 166, 1389–1415.

Demeke, T., Clear, R.M., Patrick, S.K., Gaba, D., 2005. Species-specific PCR-based assays for the detection of *Fusarium* species and a comparison with the whole seed agar plate method and trichothecene analysis. Int. J. Food Microbiol. 103, 271–284.

Gentleman, R., Ihaka, R., 1997. http://www.r-project.org/. Vienna University of Economics and Business Administration.

Hudec, K., Muchová, D., 2010. Influence of temperature and species origin on *Fusarium* spp. and *Microdochium nivale* pathogenicity to wheat seedlings. Plant Protect. Sci. 46, 59–65.

Isebaert, S., De Saeger, S., Devreese, R., Verhoeven, R., Maene, P., Heremans, B., Haesaert, G., 2009. Mycotoxin-producing *Fusarium* species occurring in winter wheat in Belgium (Flanders) during 2002–2005. J. Phytopathol. 157, 108–116.

Köhl, J., de Haas, B., Kastelein, P., Burgers, S.L.G.E., Waalwijk, C., 2007. Population dynamics of *Fusarium* spp. and *Microdochium nivale* in crops and crop residues of winter wheat. Phytopathology 97, 971–978.

Koch, H.J., Pringas, C., Maerlaender, B., 2006. Evaluation of environmental and management effects on Fusarium head blight infection and deoxynivalenol concentration in the grain of winter wheat. Eur. J. Agron. 24, 357–366.

Kosiak, B., Torp, M., Skjerve, E., Thrane, U., 2003. The prevalence and distribution of *Fusarium* species in Norwegian cereals: a survey. Acta Agricult. Scand. B 53, 168–176.

Leonard, K.J., Bushnell, W.R., 2003. Fusarium Head Blight of Wheat and Barley. APS Press, St. Paul, Minnesota, 512 pp.

Pereyra, S.A., Dill-Macky, R., 2008. Colonization of the residues of diverse plant species by *Gibberella zeae* and their contribution to Fusarium head blight inoculum. Plant Dis. 92, 800–807.

Pereyra, S.A., Dill-Macky, R., Sims, A.L., 2004. Survival and inoculum production of *Gibberella zeae* in wheat residue. Plant Dis. 88, 724–730.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A., Allard, R.W., 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. U. S. A. 81, 8014–8018.

Schaafsma, A.W., Hooker, D.C., 2007. Climatic models to predict occurrence of *Fusarium* toxins in wheat and maize. Int. J. Food Microbiol. 119, 116–125.

Waalwijk, C., Kastelein, P., de Vries, I., Kerényi, Z., van der Lee, T., Hesselink, T., Köhl, J., Kema, G., 2003. Major changes in *Fusarium* spp. in wheat in the Netherlands. Eur. J. Plant Pathol. 109, 743–754.

Wagacha, J.M., Muthomi, J.W., 2007. *Fusarium culmorum*: infection process, mechanisms of mycotoxin production and their role in pathogenesis in wheat. Crop Prot. 26, 877–885.

Weeda, E.J., Adema, F.A.C.B., Van Der Meijden, R., 1983. Flora van Nederland, twentieth ed. Wolters-Noordhoff, Groningen, 583 pp.

Xu, X.M., Parry, D.W., Nicholson, P., Thomsett, M.A., Simpson, D., Edwards, S.G., Cooke, B.M., Doohan, F.M., Brennan, J.M., Moretti, A., Tocco, G., Mule, G., Hornok, L., Giczey, G., Tatnell, J., 2005. Predominance and association of pathogenic fungi causing Fusarium ear blight in wheat in four European countries. Eur. J. Plant Pathol. 112, 143–154.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for growth stages of cereals. Weed Resistance 14, 415–421.